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9/14/11

VDS

**PyMol Refresher**

***Objective:***

*To* examine three dimensional structure of a new enzyme

DHFR-TS from *Trypanosoma cruzi* is a bi-functional enzyme complex that carries out the role of dihydrofolate reductase and thymidylate synthase. *T. cruzi* is the pathogen responsible for Chagas disease (also called American trypanosomiasis), which causes approximately 50,000 deaths annually. The disease is endemic in South and Central America. In the chronic form, Chagas disease causes severe damage to the heart and other organs. There is no satisfactory treatment for chronic Chagas disease and no vaccine is available. Potentially, this target could be used to inhibit growth of the parasite.

For each protein, make a differently colored selection for each of these categories: hyfo = hydrophobic, pol = polar, and ion = ionic. See the Spring lab PyMol 2 for specifics.

Save your session(s) as .pse session files. Remember it is nice to incrementally save your PyMol sessions so that you don’t have to start from scratch – e.g. save it as a new name every ten minutes or so.

***Materials:***

Files Used

* 2H2Q.pdb
* 3CL9.pdb
* 1U72.pdb
* 3HBB.pdb

**2H2Q** *Time: 4:23*

* This is the PDB identifier for the complex with the natural substrates. Make a PyMol image showing all of the components separately (each component should be selected individually and given a name). Display each chain distinctively. Show polar contacts between the protein and any substrates or cofactors.

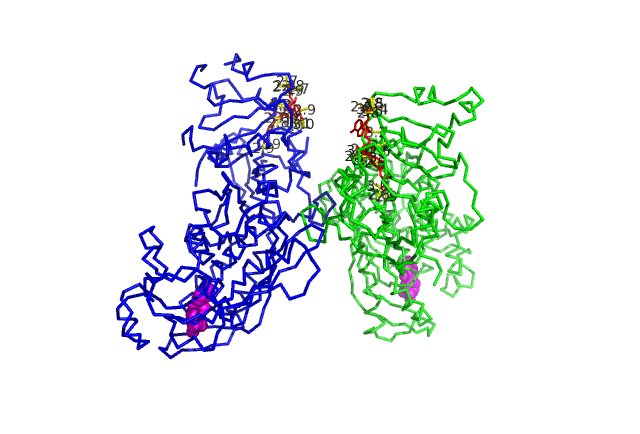


Image 1: 2H2Q show as two separate chains. Chain 1 (blue) and Chain 2 (green) are shown as ribbons. NAP are the two substrated and are shown in red as sticks. The polar contacts are also shown as dashed lines in yellow. The two cofactors in each chain are shown as spheres in pink.

**3CL9** *Time: 4:49*

* This is the PDB identifier for the A chain (DHFR portion) with a known inhibitor (MTX). Make an image showing all of the components and then the polar contacts between the inhibitor and protein. Highlight the active site (around 5 angstroms from ligand) in a different color.
* $select active, MTX around 5 #code to select active site around object ‘MTX’

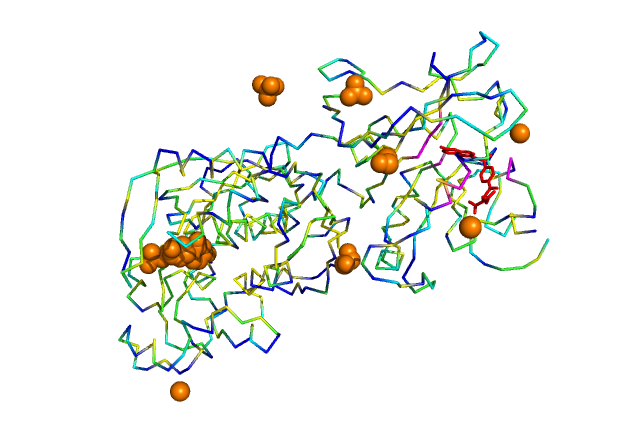


Image 2: 3CL9 shown as ribbon. The MTX is shown in red as sticks. It is inhibiting the active site (pink) of the protein. The other cofactors are shown as orange spheres.

**1U72** *Time: 5:16*

* 1U72 – This is the PDB identifier for Human DHFR with MTX. Create selections for all of the components. Then, highlight the active sites (around 5 angstroms from ligand) in different colors. Then, bring the two proteins together by performing an alignment to show how closely the *T. cruzi* and the *H. sapiens* structures line up (compare 1U72 and 3CL9). Record the RMS value. How close are the binding modes of MTX to each of these two enzymes? Do you think the enzymes could be differentially targeted with a single drug? Are there any differences in the amino acid sequence in the active site (i.e. amino acids that come in contact with the ligand)? To determine the active site, click on your active site selection and then copy down the amino acids that are highlighted in the Sequence Viewer above. Show a pairwise comparison of the active site sequences in your report. Are there any similarities/differences between them?

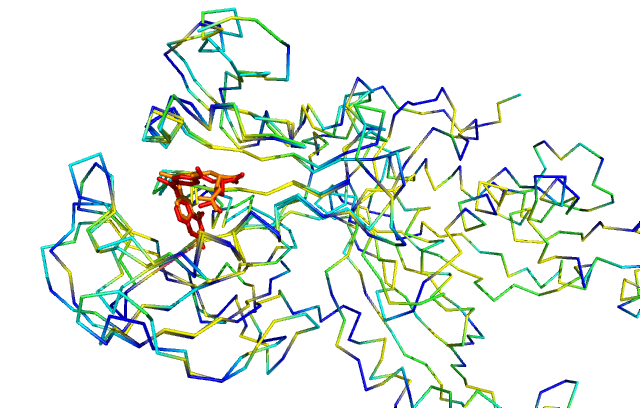


Image 3: 3CL9 and 1U72 superimposed with each other. The MTX from *H. sapiens* is shown in orange and the MTX from *T.Cruzi* is shown in red. . The RMS value is 1.147. The active sites are shown in yellow and are almost identically aligned. The binding modes of MTX for these two enzymes are pretty much identical with only slight deviations in the rings at one end of the MTX. The main portion of the MTX that is found in the active site over are superimposed almost identically. The enzymes could not be be differentially targeted since the MTX binding modes are so similar. Therer are a few differences in amino acids between the two enzymes as the human active site include leucine, glutamine, and serine while the *T.Cruzi* active site contains methionine and lysine. The pairwise comparison shows how the active sites are similar (yellow) and how they vary (pink) in amino acids as highlighted below.

***Pairwise comparison:***

>lcl|10919 unnamed protein product

Length=521

Score = 78.2 bits (191), Expect = 2e-19, Method: Compositional matrix adjust.

Identities = 55/170 (32%), Positives = 84/170 (49%), Gaps = 25/170 (15%)

Query 3 SLNCIVAVSQNMGIGKNGDLPWPPLRNEFRYFQRMTT------TSSVEGKQNLVIMGKKT 56

+ + +VAV + GIG +PW + + ++F+ +TT K+N V+MG+KT

Sbjct 22 AFSLVVAVDERGGIGDGRSIPWN-VPEDMKFFRDVTTKLRGKNVKPSPAKRNAVVMGRKT 80

Query 57 WFSIPEKNRPLKGRINLVLSRELK----------EPPQGAH-----FLSRSLDDALKLTE 101

W SIP K RPL GR+N+VLS L E + H ++ L+ AL+L

Sbjct 81 WDSIPPKFRPLPGRLNVVLSSTLTTQHLLDGLPDEEKRNLHADSIVAVNGGLEQALQLLA 140

Query 102 QPELANKVDMVWIVGGSSVYKEAMNHPG-HL--KLFVTRIMQDFESDTFF 148

P ++ V+ +GG SVY EA+ P HL ++ T I S + F

Sbjct 141 SPNYTPSIETVYCIGGGSVYAEALRPPCVHLLQAIYRTTIRASESSCSVF 190

**3HBB** *Time: 5:28*

* 3HBB – This is the PDB identifier for the complex with another known inhibitor (TMQ). Make a PyMol image showing all of the components and polar contacts between the protein and inhibitor. Display each chain distinctively. Highlight the active site (around 5 angstroms from ligand) in a different color. Is the binding mode of TMQ to *T. cruzi* DHFR-TS significantly different than that of MTX to human DHFR from 1U72? Address polar contacts and relevant amino acids in the active site.

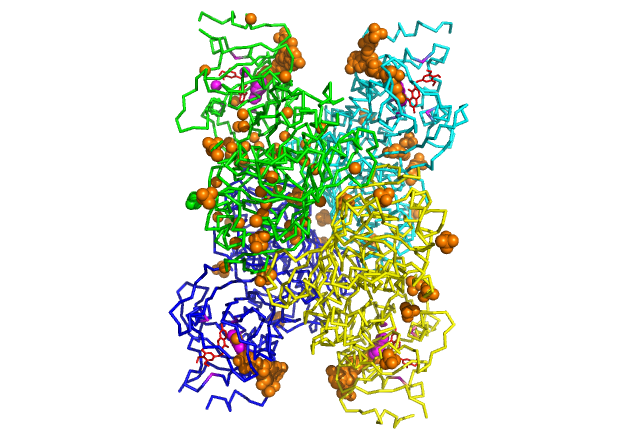
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Image 4: The four chains are shown as ribbon sin green, yellow, blue, and cyan. The active sites are shown in pink. The TMQ inhibitors are all shown as sticks in red with the polar contacts present as well. Alanine and valine are present in all 4 active sites. No the binding mode of TMQ to *T. cruzi* DHFR-TS is not significantly different than that of MTX to human DHFR from 1U72

Completed the lab at 6:00

*Conclusion:*

The lab was completed in a timely fashion. No errors seemed to be made as of now. PyMol was rather easy to use as it was a good refresher from last semester.

*Immediate Next Step:*

Continue to use PyMol to be better adjusted to the program for further advancements in research.